EXHIBIT 5

(*c*) OVIPOSITION

THE ACT OF OVIPOSITION

Mitchell (1907) and also Howard, Dyar and Knab (1912) give a translation of a description of the act of oviposition by Agramonte.

> The mosquito alighted upon the water, which was in a small beaker inside the jar, with legs spread apart. The abdominal segments being bent forwards and downwards, she dipped her whole body until the last segment touched the surface of the water; then she rose, walked on a few steps, and dipped again. This she would do repeatedly (14 to 22 times), when she would remain for a slightly longer time with the last abdominal segment touching the water, and would allow a minute egg to issue forth upon the surface. In this way she laid at the rate of 3 eggs per minute, resting quietly after every sixth or eighth egg for about 30 seconds when she would resume the process.

Fielding (1919) states that the favourite position is resting on the side of the receptacle with the tip of the abdomen just touching the water. Eggs were often laid on the water as females moved about on it. Up to fifteen eggs may be laid in a batch.

Owing to the habit of *A. aegypti* laying eggs a few at a time at intervals and the inconspicuousness of the newly laid eggs, which are almost invisible on white filter paper, it is not easy to observe females in the act of oviposition. It has, however, been observed on a number of occasions. On most of these the female was one isolated in a specimen tube. After remaining for some days, following upon feeding, almost entirely at rest near the top of the filter paper slip or on the muslin cover of the tube the insect shortly before ovipositing begins to move about in a restless way. Shortly after this ovipositing begins.

The insect during the act has much the same attitude as when resting, but the abdomen is from time to time bent downwards in a curved manner and is moved about to touch the wet surface. Each time the tip touched the surface an egg was seen to have been deposited. The action was a rather unexpected one from the deliberate manner in which the abdomen was used first to touch one place and then another, much as an elephant might use its trunk. Without the body moving, eggs were laid at spots 2 or 3 mm. apart, the abdomen moving laterally quite distinctly to do this. After laying four or five eggs the body was often raised and the abdomen straightened out, whilst the apex was vigorously rubbed by the hind-legs. The abdomen was then lowered as before and the depositing of eggs continued. After laying a group of about twenty eggs the female in each case moved to another part of the tube. The eggs were found to be adherent to the glass when the film of liquid was dried off.

SELECTION OF A SITE FOR OVIPOSITION

Two issues are here concerned, namely (1) choice of the type of site, and (2) choice of the position where the eggs are to be laid. The first relates to the type of breeding place selected by the insect and has already been discussed when dealing with the nature of the breeding places made use of by the species. The second is concerned with exactly where in the chosen receptacle the female places her eggs. It is mainly the second issue which can be studied in the laboratory and with which we are here concerned.

In the laboratory *A. aegypti* at full maturation of the ovaries will, under almost any conditions, deposit its eggs freely on any water or wet surface that may be available. It appears never to lay on a dry surface and in the absence of water or a wet surface will hold

its eggs.* The wet surface may be glass, earthenware, filter paper, sponge or the floor of the cage wet with seeped or spilled water.

They may be laid (1) on the surface of the water; (2) at the edge of the water, almost invariably on the wet surface just above the edge. Which of these sites is chosen depends largely upon the nature of the receptacle. If this is of glass, the majority of the eggs are laid on the water. If of unglazed earthenware, they are laid along the edge. Hence if floating eggs are required, a glass pot should be placed in the cage and vice versa. Howard, Dyar and Knab say that *A. aegypti* lays on the surface of the water only in captivity, glass being an unsuitable surface. Probably glass is too smooth to give the foothold that earthenware gives. That in the case of the earthenware pot both water and wet earthenware surface are available points to the fact that the insects prefer the latter. The accumulation of a line of eggs at the water's edge is not as sometimes thought the result of eggs being drawn into this position by capillarity. Actually *Aëdes* eggs are not very apt to be drawn to the edge by capillarity, though empty shells and collapsed eggs are. The eggs are so laid *in situ* by the female and are retained in position by their sticky chorionic pads until when dry they are firmly cemented in place by this same structure.

Obtaining eggs on wet filter paper may be a convenient method for enabling small samples of eggs to be used in experimentation. Floating eggs collected on cover-glasses or slides may also be useful for some purposes. But as noted in the section on technique the most suitable method and one with many advantages for obtaining and storing eggs is by the use of unglazed earthenware pots. Also, it may be noted from what has been said about hatching that filter paper, where eggs are employed for critical hatching data, requires to be used with much caution. Recently the effect of the physical characters of the container on oviposition and the desirability of using a rough porous surface have been emphasised by O'Gower (1955) in respect to *A. scutellaris*.

Eggs are invariably laid with the ventral surface, that is the surface furnished with the peculiar refractile bodies, upwards. When not over-crowded they are mostly laid in lines. Very often inspection shows that the line has followed a streak in the glass, or that the eggs have been laid along the edge of the filter paper strip where this meets the glass. In this case the eggs are almost invariably cemented to the glass, not to the paper. Usually the eggs are laid lenthwise in the direction of the line, but not necessarily all pointing in the same direction.

A point frequently referred to in the literature is the question of the choice for oviposition of the suitable fluid. It is commonly stated that this is by preference water containing organic matter, as against clean water. Beattie (1932) found the largest number of eggs on test bottles containing leaf infusion (8309 eggs from forty-two layings, as against 1241 eggs with twenty-nine layings with distilled water). In the laboratory, however, females appear to lay without any hesitation on clean tap-water and as a routine our pots have always been boiled and well scrubbed and placed in the cage with fresh clean tap-water. There are certain advantages in cleanliness in this respect and, to avoid unnecessary fouling by pre-oviposition defaecation, the pots for oviposition are freshly changed after this has taken place.

It is doubtful indeed if there is any very marked selection of the character of the water if other conditions are suitable, except in respect to strong salinity or other objectionable feature. Fielding, for example, notes that eggs may be laid on 40 per cent sea-water. Wallis (1954), studying the oviposition activity of mosquitoes including *A. aegypti*, found

* Incidentally if it did so the eggs would certainly perish; see description of changes following oviposition in ch. VI (*d*).

that the female could still detect an objectionable amount of salt when the movements of the abdomen were restricted and the palps, proboscis and antennae were coated with wax, and considered that, in general, sensitivity was located in the tarsal segments (see ch. XXIX).

RELATION OF BLOOD MEAL TO FERTILISATION AND OVARIAN DEVELOPMENT

The blood meal has little or no relation to copulation or fertilisation. Marchoux, Salimbeni and Simond state that a blood meal is not necessary to fertilisation. Macfie (1915) states that fertilisation can take place without feeding. Howard (1923) says that virgin females will feed, but that fertilised females are more greedy. Brug (1928) states that unfertilised females suck blood as readily as fertilised and, contrary to the statement by Macfie, (1915) they also lay eggs (unfertilised).

That for ovulation a blood meal is necessary is now generally accepted. It seems to be immaterial at what stage of life the blood meal is taken. Bacot records that a female fed 56 days on honey and white of egg laid fertile eggs 4 days after the first blood meal. Fielding, who failed to obtain eggs from females fed on a variety of foods other than blood, also found that egg production ceased on substitution of banana for blood. Gordon (1922) offered fifty-four females serum, washed red cells and whole blood and found eggs produced only on the last-mentioned.

That more than one blood meal is necessary before *A. aegypti* can develop its eggs has been sometimes stated. Whilst this is obviously incorrect where a full blood meal has been taken it raises the question how much blood is necessary. This has been very fully investigated by Roy (1936) whose work on the weight of blood taken at a meal has already been referred to. This author compared the number of eggs which were laid following imbibition of different amounts of blood. The data given in Table 36 are taken from his paper.

Table 36. *Number of eggs laid following upon different amounts of blood feed (from data given by Roy)*

Range blood (mg.)	Number of females	Mean weight blood (mg.)	Total number of eggs laid	Mean number of eggs laid	Number of times no eggs laid	Number of eggs per mg.
0·0–0·5	21	0·38	0	0	21	—
0·6–1·0	28	0·77	197	7·04	21	9·14
1·1–1·5	35	1·22	1197	34·2	2	28·06
1·6–2·0	34	1·79	1699	47·03	1	27·77
2·1–2·5	11	2·17	544	49·45	1	22·71
2·6–3·0	6	2·72	404	67·33	0	24·77
3·1–3·5	2	3·2	171	85·5	0	26·72

The table shows very clearly that to obtain a mean of 85·5 eggs, which it will later be shown is about the normal, a full meal of 3·0–3·5 mg. blood is required and that for any eggs at all at least 0·5–1·0 mg., or on the average 0·8 mg., of blood is necessary.

Further, small amounts of blood in successive feeds were capable of producing eggs only when the weight of blood taken at the third feed exceeded 0·5 mg. The number of eggs under such conditions was, however, as a rule small. In one batch mosquitoes given feeds from 0·13 to 0·45 mg. produced no eggs. But seven females given over 0·47 all produced some eggs. The author considered that these minimal amounts were necessary to stimulate the ovaries and that excess over this goes to form eggs. In chapter XXXI in

SPECIAL SENSES AND BEHAVIOUR

In regard to temperature Hecht (1930) found that *A. aegypti* laid most eggs between 20° and 30° C., but the temperature choice was less sharply defined than with *Anopheles*. Oviposition, according to Gutzevich (1931), does not occur below 16° C. With the present author it has taken place at all temperatures between 19° and 28° C., the only difference noted being some spreading of the time taken at the lower temperatures.

There are many references in the literature to the varying attractiveness of different media for oviposition by *Aëdes aegypti*. Buxton and Hopkins (1927) found infusions of hay, rice or bran very attractive for females in nature. There has never been in our case any hesitation of females in captivity in laying on or around clean tap-water. The masses of eggs, amounting to 10,000 or more in a pot, were laid on clean water and the pots for technical reasons were perfectly clean and free from organic matter and newly placed in position after fouling by the pre-oviposition defaecation. More important than the nature of the fluid, provided this is not objectionable, is the nature of the receptacle (see under 'Selection of site for oviposition', ch. XXII (*c*), p. 504).

In regard to salinity Macfie (1915) notes that *A. aegypti* shows a selective choice for tap-water over salt solutions and that females will not oviposit at all on 2 per cent salt, or will do so only after much delay. Fielding (1919) found eggs laid on 70 per cent sea-water (about 2 per cent salt), but in higher concentrations none were laid. Woodhill (1941) states that *A. aegypti* distinguishes between water containing 5, 10, 17·5 and 35 parts per thousand of salt and will not lay on water containing 35 parts. Roubaud and Colas Belcour (1945) note that all types of water, tap, pure, distilled, or diluted sea-water are utilised with no particular preference, though eggs on sea-water remained white and the chorion did not harden (see ch. VI (*d*)). Of 17,940 eggs laid 28·9 per cent were on tap-water, 28 per cent on 31 per cent sea-water, 22·2 per cent on 42·6 per cent sea-water, 17·8 on 62 per cent sea-water and 3·1 per cent on undiluted sea-water. These authors consider that the only influence affecting oviposition is hygrotropism, which affects both males and females and apparently is governed by a special sensibility of the tip of the abdomen in respect to unfavourable substances in the medium. Howlett (1919) found sodium citrate and tartrate had an attractive effect for oviposition of *A. albopictus*.

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